UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

October 30, 2014

MEMORANDUM

Subject: Protocol Review for 42182PA3 (Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity for Hospital Use); DB Barcode: D422195.

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Applicant: Microban International, Ltd.
11400 Vanstory Drive
Huntersville, NC 28078.

I. BACKGROUND

Microban International, Ltd. intends to conduct an analysis of dried product efficacy, following EPA Protocol # 01-1A and guidance received from the Antimicrobial Division's Efficacy Team. Through the current submission, the registrant is submitting a bacterial efficacy protocol to support residual self-sanitization/disinfection claims for hospital use for a liquid/spray disinfectant product (Microban Firebird 127 Disinfectant). Protocol was developed by Antimicrobial Test Laboratories (ATL), located at 1304 W. Industrial Blvd., Round Rock, Texas 78681.

This data package is identified as D422195 contained a letter from the applicant's representative (dated July 15, 2014), and one protocols (MRID no. 494316-01).

II. BRIEF DESCRIPTION OF THE PROTOCOL

MRID 494316-01

Title:
Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity for Hospital Use

Purpose:
The purpose of this study is to document the residual activity of the test substance against the test systems (microorganisms) under the test parameters specified in this protocol

Active Ingredient Concentration

Method References:
EPA Protocol # 01-1A. Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces.

Test System (Microorganism):

Staphylococcus aureus ATCC 6538
Enterobacter aerogenes ATCC 13048
Pseudomonas aeruginosa ATCC 15442

Additional Bacteria: {Insert additional strains (e.g. E. coli, MRSA)}

Required bacteria will be tested on 3 lots per organism. Additional bacteria will be tested on 2 lots per organism.

Study Parameters

Residual Self-Sanitizing / Self-Disinfecting Efficacy

Contact Time - {Insert exposure period (e.g. 5mins, 10mins, 1 hour).

For Residual Sanitizer claims, a 99.9% reduction must be demonstrated in ≤ 5 minutes ± 5 seconds.

For Residual Disinfection claims, a 99.999% reduction must be demonstrated in ≤ 10 minutes ± 5 seconds

Continuous Reduction Period - {Insert time period (e.g. 12 hours, 24 hours). The following procedure is based on a 24 hour continuous reduction period thus the procedure must be completed in >24 hours but no longer than 48 hours. If shorter or longer continuous reduction claims are desired, then the Wear, Abrasion, and Re-inoculation frequencies and the laboratory duration of the procedure must be adjusted ratiometrically. The period must not be less than 4 hours. For example to support a 12 hour continuous reduction claim, the Wear, Abrasion, and Re-inoculation frequencies described below would be reduced by 50% and the study must be completed in >12 hours but no longer than 24 hours. For example to support a 48 hour continuous reduction claim, the Wear, Abrasion, and Re-inoculation frequencies described below would be doubled and the study must be completed in >48 hours but no longer than 96 hours.}

Abrasion Control Replicates - 4 per test microorganism
Non-Abrasion Control Replicates - 4 per test microorganism
Test Surface Replicates - 4 per test substance, per test microorganism
Neutralization Controls - 2 per test substance, per test microorganism inoculum level

Exposure of Test Carriers to Test Substance

- Four test carriers (per lot, per microorganism) are treated by spray application with the test substance. Each carrier is treated according to the study sponsor's instructions.
- After treatment, the test substance on the carriers is allowed to dry at room temperature and 45-55% relative humidity with the lids ajar for up to 1 hour such that the first Wear cycle begins no later than no longer than 1 hour after treatment of the carrier.

Abrasions and Re-inoculations

- Test Carriers and Abrasion Control Carriers undergo a wear and re-inoculation regimen including a series of at least 12 wear cycles and 12 re-inoculation cycles to support a 24 hour continuous reduction claim. The Non-Abrasion Control Carriers do not undergo the wear cycling. The number, duration, and order of abrasions may be ratiometrically modified by the Study Sponsor to support the desired claim as described above. This step is performed at room temperature. The table below summarizes the manipulations of all carriers in the study.
- Abrasions are conducted between 45-55% relative humidity (RH). Temperature and room humidity measurements are taken and recorded periodically throughout the abrasion process.
- The weight of the fully assembled abrasion boats are recorded prior to initiation of the wear and reinoculation regimen and must equal 1084 ± 1.0g
- The abrasion tester is set to a speed of 2.25 to 2.5 for a total surface contact time of approximately 8-10 seconds, for one complete abrasion cycle. Each abrasion cycle in this test equals four (4) passes, one pass to the left and one return pass to the right followed by another pass to the left and another return pass to the right.
- All surfaces in contact with carriers on the abrasion tester are decontaminated with ethanol and allowed to dry completely between each set of surface wears to prevent carryover contamination.
- The foam liner and cotton cloths on the abrasion tester are replaced between each set of abrasion.

- After each complete set of abrasions are conducted (all control and test carriers abraded), the carriers are allowed to sit undisturbed for at least 15 minutes.
- Control and test carriers are then re-inoculated with 0.010ml of the re-inoculation culture and spread within 1/8 inch of the surface edge, and then allowed to dry at ambient temperature for a minimum of 30 minutes or until completely dry prior to initiation of the next set of abrasions.
- Cotton cloths used as part of wet abrasions are prepared individually prior to each wet abrasion cycle by spraying the cloth with sterile reverse osmosis water using a sanitized Preval sprayer, from a distance of 75±1cm for no more than 1 second and used immediately.

Procedure Timeline (Hours)	Abrasion/Re-inoculation Procedure	Target CFU/Carrier
0	Initial inoculation of Test and Control Carriers	10^6
1	Test Substance Application and Drying	
1-24	Dry Abrasion (wear #1)	10^4 with each reinoculation
	Re-inoculation (1)*	
	Wet Abrasion (wear #2)	
	Re-inoculation (2)*	
	Dry Abrasion (wear #3)	
	Re-inoculation (3)*	
	Wet Abrasion (wear #4)	
	Re-inoculation (4)*	
	Dry Abrasion (wear #5)	
	Re-inoculation (5)*	
	Wet Abrasion (wear #6)	
	Re-inoculation (6)*	
	Dry Abrasion (wear #7)	
	Re-inoculation (7)*	
	Wet Abrasion (wear #8)	
	Re-inoculation (8)*	
	Dry Abrasion (wear #9)	
	Re-inoculation (9)*	
	Wet Abrasion (wear #10)	
	Re-inoculation (10)*	
	Dry Abrasion (wear #11)	
	Re-inoculation (11)*	
	Wet Abrasions (wear #12)	
≥24-48	Determination of Residual activity	10^6

Success Criteria:

• The experimental success (controls) criteria follow:

1. In the Neutralization Control, test substance treated carrier counts must be within 0.50 $\log_{10}$ of the control treated carrier counts.
2. The media sterility controls are negative for growth.
3. The purity "isolation streaks" demonstrate a pure culture of test microorganism as evidenced by colony morphology.
4. The carrier sterility controls are negative for growth.

5. The soil sterility control is negative for growth.
6. The Initial Inoculation Carrier Control must have a minimum of 1 x 10^6 CPU/carrier.
7. The Re-Inoculation Carrier Control carriers must have a minimum of 1 x 10^4 CPU/carrier.
8. The Final Abrasion Control must have a minimum of 1 x 10^6 CPU/carrier.

- Test substance performance criterion for public health claims:
  - To be defined as a residual disinfectant for healthcare use, product must:
    - meet the OCSPP 810.2200 requirements for a hospital disinfectant, and
    - in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 5 $\log_{10}$ or 99.999% at a contact time of ≤10 minutes.
  - To be defined as a residual sanitizer for healthcare use, the test product must:
    - meet the OCSPP 810.2200 requirements for a hospital disinfectant, and
    - meet the OCSPP 810.2300 requirements for a non-food contact sanitizer, and
    - in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 3 $\log_{10}$ or 99.9% at a contact time of ≤5 minutes.

III. CONCLUSION AND COMMENTS

1. The submitted protocol under MRID 494316-01 **is adequate for testing** 24 hours residual bacterial activity of dried chemical on hard non-porous hospital environment surfaces. However, "Initial inoculation of Test and Control Carriers" step cannot precede "Test Substance Application and Drying" step if residual activity of dried chemical is being tested. **Registrant must revise procedure table to reflect "Test Substance Application and Drying" form 0 to 1 hour; followed by "Initial inoculation of Test and Control Carriers"**.

2. Because the protocol is being used to conduct efficacy testing of dried chemical on hospital environmental hard non-porous, **the following claims are not acceptable**:

a. Residual Self-Sanitization of dried chemical; **all references to self-sanitization must be removed**.

b. Additional bacterial residual self-sanitization or self-disinfection of dried chemical; **all references to residual self-sanitization or self-disinfection activity on additional bacteria must be removed**.

c. Continuous bacterial reduction claims exceeding 24 hours for hospital environmental hard non-porous. **All references to continuous residual self-sanitization or self-disinfection exceeding 24 hours must be removed**.

3. It is a reminder that product must be tested at the lower certified limit proposed on the CSF.

4. The potential variability in the method must be addressed prior to data generation. The Agency encourages the testing laboratory to assess the degree and sources of variability introduced by any significant method modification – this information should be supplied to the Agency prior to GLP testing. For example, preliminary runs of the study should be performed to determine the degree of variability associated with control and treated carriers; the number of carriers should be increased if the variability is too high.

5. Identify and use the most recent versions of all standard methods cited in the protocol. Specify the broth media for generating test cultures and the plating medium for recovery of each test microbe [Use the AOAC Use-dilution method for preparation of cultures of *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), or *Staphylococcus aureus* (ATCC 6538).]

6. The study controls must perform according to the criteria detailed in the protocol. If any of the control acceptance criteria are not met, the test may be repeated.

7. Provide a list of any deviation or modification to a standard method.